Accompanying Documents

1. A marked-up copy of pages 14 and 15 and the claim set indicating the amendments set forth in this paper (Appendix A);
2. A clean copy of pages 14 and 15 and the pending claims after entry of the amendments set forth in this paper (Appendix B);
3. Request for extension of time;
4. Check; and
5. Postcard.

AMENDMENT

In the Specification:

On page 15, in line 3, please delete "http://".

A marked up copy of pages 14 and 15 showing this amendment is presented in Appendix A. A clean copy of pages 14 and 15, after entry of the amendment, can be found in Appendix B.

In the Claims:

Please cancel claim 62, without prejudice or disclaimer. Please amend claims 79-82, without prejudice or disclaimer, as shown in Appendix A.

A clean copy of the claims, after entry of the amendments, can be found in Appendix B.

REMARKS

Introductory Comments

Claims 1-8, 21, 22, 24-29, 34-36, 49, 56, 58, 62, 64, and 68-82 were pending in the present application. Claims 60 and 83-86 have been withdrawn from consideration by the Examiner. Claim 62 has been canceled without prejudice or disclaimer. Claims 79-82 have been amended without prejudice or disclaimer. Cancellation or amendment of the claims is not intended to be an acquiescence in the Office's assessment of those claims in the 4 June 2002 Office action, and applicants expressly reserve the right to bring the subject matter of the original claims again in a subsequent, related application. After entry of the amendments in this paper claims 1-8, 21, 22, 24-29, 34-36, 49, 56, 58, 64, and 68-82 are pending.

The Examiner maintains the assertion that claim 60 (and newly added claims 83-86) should be restricted from Group I (i.e., the claims currently under prosecution). Applicants maintain their traverse of the restriction.

However, the Examiner notes the following:

> Claims 1-8, 21, 22, 24-29, 34-36, 49, 56, 58, 62, 64, and 68-**83** are under consideration. (Office action, dated 4 June 2002, page 3, last paragraph, emphasis added).

Further, claim 83 is rejected by the Examiner under 35 U.S.C. §112, first paragraph (Office action, dated 4 June 2002, page 4, fourth and fifth paragraphs). Applicants have proceeded with this response based on the assumption that claim 83 has been withdrawn from consideration by the Examiner. Applicants respectfully request clarification concerning whether claim 83 is pending.

In the Office action, dated 4 June 2002, the following objection and rejections were asserted by the Examiner:

The Examiner has objected to the specification for containing an embedded hyperlink and/or other form of browser-executable code.

The Examiner has rejected claims 1-3, 5-8, 21, 22, 24-28, 34-36, 49, 56, 58, 62, 64 and 68-83 under 35 U.S.C. §112, first paragraph, asserting that the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The Examiner has rejected claim 62 under 35 U.S.C. §112, first paragraph, asserting that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected how to make the invention commensurate in scope with the claims.

The Examiner has rejected claim 21, with dependent claims 22, 24-29, and 76-82 under 35 U.S.C. §112, second paragraph, asserting that the claims are indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.

The Examiner rejected claims 1, 3, 14, 30, 32, 33, 37, 48, 49, 59, 67, 69, 70, 75, and 76 under 35 U.S.C. §103(a) asserting that the claims are unpatentable over Meighen ("Bacterial bioluminescence: organization, regulation, and application of *lux* genes," FASEB Journal **7**(11):1016-1022 (1993)), in view of Vellanoweth, et al. (Abstract only, cited by the Examiner).

The Examiner rejected claims 21, 24-29, 70-74, and 76-82 under 35 U.S.C. §103(a) asserting that the claims are unpatentable over Schauer (Abstract only, cited by the Examiner), in view of Vellanoweth, et al. (Abstract only, cited by the Examiner).

The Examiner rejected claim 22 asserting that the claim is unpatentable over Schauer (Abstract only, cited by the Examiner), in view of Vellanoweth, et al. (Abstract only, cited by the Examiner), and further in view of Meighen ("Bacterial bioluminescence: organization, regulation, and application of *lux* genes," FASEB Journal 7(11):1016-1022 (1993)).

Claims 62 (now canceled), 68, and 69 are free of the art, as none of these claims were included in any of the above-recited art-based rejections.

The objection and rejections are believed to be overcome in part by the amendments and are otherwise traversed for reasons discussed below.

Overview of the Amendments

In the Specification:

The amendment to page 15 removes the term "http://" as required by the Examiner.

Accordingly, no new matter has been added by way of this amendment and the entry thereof is respectfully requested.

In the Claims:

Support for the amendments to claims 79-82 can be found throughout the specification as filed, for example, at the following locations: page 23, line 22, to page 25, line 20; and page 32, lines 4-16.

Accordingly, no new matter has been added by way of this amendment and the entry thereof is respectfully requested.

Addressing the Examiner's Rejections

1. Objection to the Specification

The Examiner has objected to the specification for containing an embedded hyperlink and/or other form of browser-executable code.

MPEP §608.01 states the following:

> When a user clicks on the link with a mouse, the user will be transferred to another web page identified by the URL, if it exists, which could be a commercial web site. USPTO policy does not permit the USPTO to link to any commercial sites since the USPTO exercises no control over the organization, views or accuracy of the information contained on these outside sites." The URL in question (i.e., http://www.ncbi.nlm.gov/cgi-bin/BLAST) is not a commercial web site. In fact, it is a website controlled by other U.S. Government Agencies (National Library of Medicine/National Institutes of Health).

In an effort to facilitate prosecution, applicants have amended the specification to remove the "http://" term from the specification. MPEP §608.01 states the following:

> Examples of a hyperlink or a browser-executable code are a URL placed between these symbols "< >" and http:// followed by a URL address.

The remaining URL address is neither placed between the "<>" symbols nor does it contain the "http://" term.

In view of applicants' amendment to the specification, applicants submit that the specification complies with the requirements of MPEP §608.01. Accordingly, applicants respectfully request withdrawal of the objection to the specification.

2. Rejection of the Claims Under 35 U.S.C. §112, First Paragraph (Written Description)

The Examiner has rejected claims 1-3, 5-8, 21, 22, 24-28, 34-36, 49, 56, 58, 62, 64 and 68-83 under 35 U.S.C. §112, first paragraph, asserting that the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

In the rejection the Examiner asserts the following:

> The specification does not contain any disclosure of the structure and function of all DNA sequences that encode a *lux* polypeptide or portions thereof. The genus of DNAs that comprise these above DNA molecules is a large variable genus with the potentiality of encoding many different proteins. Therefore, many structurally and functionally unrelated DNAs are encompassed within the scope of these claims, including partial DNA sequences. The specification discloses only a single species of the claimed genus, a DNA encoding *lux* gene products from Gram-negative bacterium *Photorhabdus (Xenorhabdus) luminescens*. Moreover, the specification fails to describe any other representative species by identifying

characteristics or properties other than the "functionality" of encoding a *lux* polypeptide and fails to provide any structure:function correlation present in all members of the claimed genus. (Office action, page 5, first full paragraph).

First, applicants are not required in their specification to describe "all DNA sequences that encode a *lux* polypeptide" nor "all members of the claimed genus." MPEP 2163 (Guidelines for the Examination of Patent Applications Under the 35 U.S.C. §112, para. 1, "Written Description" Requirement) states the following:

> The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice (see i)(A), above), reduction to drawings (see i)(B), above), or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus (see i)(C), above). See Eli Lilly, 119 F.3d at 1568, 43 USPQ2d at 1406...
>
> A "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. On the other hand, there may be situations where one species adequately supports a genus...
>
> What constitutes a "representative number" is an inverse function of the skill and knowledge in the art. Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed...
>
> Description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces.

Second, as stated in MPEP 2163 (Guidelines for the Examination of Patent Applications Under the 35 U.S.C. §112, para. 1, "Written Description" Requirement):

> What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. See Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d at 1384, 231 USPQ at 94. If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate

description requirement is met. See, e.g., Vas-Cath, 935 F.2d at 1563, 19 USPQ2d at 1116; Martin v. Johnson, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972) (stating "the description need not be in ipsis verbis [i.e., "in the same words"] to be sufficient").

Applicants note in the Background of the Invention:

> Bioluminescent bacteria are widely found in both marine and terrestrial environments. Interestingly, all identified species of naturally occurring marine and terrestrial bioluminescent bacteria are Gram-negative. To date, at least eleven species in four Gram-negative genera have been described: *Vibrio*, *Photobacterium*, *Shewanella* (*Altermonas*) and *Photorhabdus* (*Xenorhabdus*). In all these species, the five genes responsible for bioluminescence are clustered in the *lux* operon (*luxCDABE*).
>
> The bioluminescence (emitted blue-green light having a wavelength of about 490 nm) is thought to result from a luciferase-catalyzed oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long-chain fatty aldehyde. The luciferase enzyme is encoded by two subunits (*luxAB*), whereas the fatty acid reductase polypeptides responsible for the biosynthesis of the aldehyde substrate for the luminescent reaction are encoded by the three genes *luxCDE*. The genes encoding luciferase and the fatty acid reductase polypeptides have been cloned from the *lux* operons of *Vibrio*, *Photobacterium* and *Photorhabdus* and sequenced. In each case, the *luxCDE* genes flank the *luxAB* genes, with transcription in the order *luxCDABE*. Although a number of additional *lux* genes have been identified in each of these three bacteria, only *luxA-E* are essential for the biosynthesis of light (reviewed by Meighen, E., (1993,*The FASEB Journal* **7**:1016-1022 and Ulitzur, S., (1997), *J. Biolumin Chemilumin* **12**:179-192). (Specification, page 1, lines 8-24).

The reference of Meighen (1993) cited by the Examiner in the rejections under 35 U.S.C. §103(a) states the following:

> Significant advances have been made in the characterization of luciferases and other *lux*-specific proteins as well as the *lux* genes from a number of different species of marine and terrestrial luminescent bacteria. A common *lux* gene organization (*lux*CDAB..E) modulated by the presence of specific genes involved in regulation and flavin binding and metabolism (*lux*F-I,L,R,Y) has been found with the luciferase genes (*lux*AB)flanked by the genes involved in synthesis of its fatty aldehyde substrate (*lux*CDE). (Abstract)
>
> Luciferase and other enzymes involved in the luminescent (*lux*) system as well as the corresponding *lux* genes have been isolated from marine bacteria in the *Vibrio* and *Photobacterium* genera and from terrestrial bacteria in the *Xenorhabdus* genus. The most widely

studied of these bacteria are the *Vibrio harveyi, Vibrio fischeri, Photobacterium phosphoreum, Photobacterium leiognathi,* and *Xenorhabdus luminescens* species. However, other species of luminescent bacteria are of interest including light-emitting *Vibrio cholerae* strains found in freshwater and the aerobic *S. hanedai* species from the *Shewanella* genus." (paragraph bridging cols. 1-2 on page 1016)

Accordingly, applicants have set forth structure:function characteristics (i.e., "the luciferase enzyme is encoded by two subunits (*luxAB*)," and "the fatty acid reductase polypeptides responsible for the biosynthesis of the aldehyde substrate for the luminescent reaction are encoded by the three genes *luxCDE*"). Applicants also note, regarding this structure:function relationship, that "(t)o date, at least eleven species in four Gram-negative genera have been described: *Vibrio*, *Photobacterium*, *Shewanella* (*Altermonas*) and *Photorhabdus* (*Xenorhabdus*). In all these species, the five genes responsible for bioluminescence are clustered in the *lux* operon (*luxCDABE*)." Further, these teachings are supported by the teachings of Meighen (for example, those presented above).

The Examiner, however, has only presented a series of unsubstantiated assertions to support the rejection. Further, the standard being set by the Examiner (e.g., "all DNA sequences that encode a *lux* polypeptide" and "all members of the claimed genus") is not the appropriate standard for examination as set forth by MPEP 2163, and case law recited therein, to meet the requirements of adequate written description.

In view of the above-arguments, applicants submit that the rejection of the claims under 35 U.S.C. §112, first paragraph (Written Description) is inappropriate. Applicants respectfully request withdrawal of this rejection of the claims.

3. Rejection of the Claims Under 35 U.S.C. §112, First Paragraph (Scope)

The Examiner has rejected claim 62 under 35 U.S.C. §112, first paragraph, asserting that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected how to make the invention commensurate in scope with the claims.

In the rejection, the Examiner asserts:

> The specification teaches that "none of the bioluminescent Gram-positive bacteria which have been published to date produce enough light" (page 2, lines 22-23). The state of the art is such that it is a priori unpredictable how the cassette

should be constructed in order to have the requisite characteristics. (Office action, dated 4 June 2002, page 6, second full paragraph.)

The teaching being relied upon by the Examiner is a statement in the Background of the Invention. In their specification the applicants have taught how to make and use the presently claimed invention. Applicants provide numerous teachings concerning, for example, sources of *lux* operon genes (for example, page 1, lines 8-24; and pages 23-24 of applicants' specification), and modification of the genes comprising the operon to improve expression in Gram-positive bacteria (e.g., pages 24-26; and the Examples of applicants' specification). Standard vectors and methods for the transformation of Gram-positive bacteria are well known in the art. The specification describes the construction and screening of luciferase-encoding vectors of the present invention (e.g., pages 19-23; pages 27-29 ; and the Examples of applicants' specification). One of ordinary skill in the art, following the guidance of the present specification combined with what is known in the art, would clearly be able to make and use the claimed invention.

The law does not require an applicant to describe in his specification every conceivable embodiment of the invention. *SRI International v. Matsushita Elec. Corp. of America*, 775 F.2d 1107, 227 USPQ 577 (Fed. Cir. 1985). Further, the enablement requirement may be satisfied even though some experimentation is required. *Hybritech Inc. v. Monoclonal Antibodies*, 802 F.2d at 1367, 231 USPQ 81 (Fed. Cir. 1986).

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*Ex parte Forman*, 230 USPQ 546 (P.T.O. Bd. Pat. App. & Int., 1986).

The Examiner has not objected that one of ordinary skill in the art could not recreate the invention. Moreover, the Examiner has merely asserted, and has not shown, that an undue amount of experimentation would be necessary to replicate the claimed invention. For example, the Examiner asserts that "(w)hile recombinant and mutagenesis techniques are known, it is not routine in the art to screen a large number of possible combinations." (Office action, page 7, first full paragraph); but no documentation to support this position is provided. Whenever the PTO makes such a rejection for failure to teach and/or use the invention, the PTO must explain its reasons for the rejection and support the rejection with (i) acceptable evidence, or (ii) reasoning which contradicts the applicants' claim: the reasoning must be supported by current literature as a

whole and the PTO must prove the disclosure requires undue experimentation. *In re Marzocchi*, 439 F.2d 220, 223-24, 169 USPQ 367, 369-70 (CCPA 1971). The Examiner has provided no such support for the present rejection.

However, in an effort to facilitate prosecution, applicants have cancelled claim 62.

In view of the above arguments and amendments, the applicants submit that the claims are enabled and that the rejection of the claims under 35 U.S.C 112, first paragraph, should be withdrawn.

4. Rejection of the Claims Under 35 U.S.C. §112, Second Paragraph

The Examiner has rejected claim 21, with dependent claims 22, 24-29, and 76-82 under 35 U.S.C. §112, second paragraph, asserting that the claims are indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The Examiner has asserted the following specific deficiencies in claims 21 and 82.

A. Claim 21

In the Office action, dated 4 June 2002, the Examiner asserts the following: "Claim 21 recites 'luc' gene that is not defined in the specification." (Office action, page 7, last paragraph.)

Contrary to the Examiner's assertion the term "luc" is defined in the specification (e.g., page 18, lines 19-24). Further, eucaryotic luciferase encoding genes (commonly designated as "luc") are well known in the art and are described in the specification, for example, at the following locations: page 22, lines 5-15; page 23, line 27, to page 24, line 8.

Accordingly, applicants submit that the rejection of claim 21, and dependent claims, is improper. Applicants respectfully request withdrawal of the rejection.

B. Claim 82

In the Office action, dated 4 June 2002, the Examiner asserts the following: "Claim 82 recites 'said substrate is (i) aldehyde and is provided as a vapor, and (ii) a substrate for the *luc* gene product (emphasis added). It appears that some words are missing."

Applicants thank the Examiner for the Examiner's attention to the language of the claims. Claim 79 has been amended to recite "one or more substrates." Claim 82 has been amended to recited "wherein said one or more substrates comprise (i) aldehyde, provided as a vapor, and (ii) a substrate for the *luc* gene product."

In view of these amendments to the claims, applicants submit that the claims comply with the requirements of 35 U.S.C. §112, second paragraph. Accordingly, withdrawal of the rejection is respectfully requested.

5. Rejections of the Claims Under 35 U.S.C. §103

The Examiner rejected claims 1, 3, 14, 30, 32, 33, 37, 48, 49, 59, 67, 69, 70, 75, and 76 under 35 U.S.C. §103(a) asserting that the claims are unpatentable over Meighen ("Bacterial bioluminescence: organization, regulation, and application of *lux* genes," FASEB Journal **7**(11):1016-1022 (1993)), in view of Vellanoweth, et al. (Abstract only, cited by the Examiner).

The Examiner rejected claims 21, 24-29, 70-74, and 76-82 under 35 U.S.C. §103(a) asserting that the claims are unpatentable over Schauer (Abstract only, cited by the Examiner), in view of Vellanoweth, et al. (Abstract only, cited by the Examiner).

The Examiner rejected claim 22 asserting that the claim is unpatentable over Schauer (Abstract only, cited by the Examiner), in view of Vellanoweth, et al. (Abstract only, cited by the Examiner), and further in view of Meighen ("Bacterial bioluminescence: organization, regulation, and application of *lux* genes," FASEB Journal **7**(11):1016-1022 (1993)).

A. Applicants Request Proper Consideration of What Each Reference Teaches as a Whole.

Applicants submit that the Examiner's rejection of the claims is improper. The Examiner has employed a primary reference (Schauer, in the rejection of independent claim 21) and a secondary reference (Vellanoweth, et al., in the rejection of independent claim 1) wherein the Examiner has only relied on the teachings of the abstract of each paper. The Examiner has neither provided complete copies of the references to the applicants nor addressed the teachings of these references as a whole.

The Examiner has failed to comply with Office policy to follow *Graham v. John Deere Co.* (383 USC 1, 86 S. Ct. 684, 15 L Ed2d 545, 148 USPQ 459, S.C., 1966) in the consideration and determination of obviousness under 35 U.S.C. §103 (see M.P.E.P. 2141, Eighth Edition). The four factual inquires enunciated in *Graham v. John Deere Co.* as a background for determining obviousness are as follows:

(A) Determining the scope and contents of the prior art;
(B) Ascertaining the differences between the prior art and the claims in issue;

(C) Resolving the level of ordinary skill in the pertinent art; and
(D) Evaluating evidence of secondary considerations.

By not setting forth rejections that clearly show the differences between the cited references and the claims at issue, in particular the differences between each primary reference and the claimed invention, followed by the teachings of the secondary references as applied to make up for the deficiencies of each primary reference, the Examiner has failed to meet the criteria for a determination of obviousness.

Applicants submit that the Examiner has not given fair weight to what each reference teaches in its entirety. The Examiner has not addressed the differences between the prior art and the claims at issue (*Graham v. John Deere Co.,* 383 USC 1, 86 S. Ct. 684, 15 L Ed2d 545, 148 USPQ 459, S.C., 1966).

B. The Examiner Has Failed to Establish a Case of *Prima Facie* Obviousness.

The Patent and Trademark Office has the burden of establishing a case of *prima facie* obviousness. Per M.P.E.P. 2141 (Eighth Edition):

> When applying 35 U.S.C. §103, the following tenets of patent law must be adhered to:
>
> (A) The claimed invention must be considered as a whole;
>
> (B) The references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination;
>
> (C) The references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and
>
> (D) Reasonable expectation of success is the standard with which obviousness is determined.
>
> *Hodosh v. Block Drug Co., Inc.,* 786 F.2d 1136, 1143, 229 USPQ 182, 187, Fed. Cir. 1986.

Further, according to M.P.E.P. 2142 (Eighth Edition):

> The examiner bears the initial burden of factually supporting any *prima facie* conclusion of obviousness. If the examiner does not produce a *prima facie* case, the applicant is under no obligation to submit evidence of nonobviousness.

In the present case, the Examiner has clearly failed to establish a *prima facie* case of obviousness because the Examiner has failed to provide any reference that teaches or suggests all of the elements of the independent claims, for example, "Gram-positive ribosome-binding site

sequences are located 5' to all of said *lux* coding sequences" (wherein the *lux* coding sequences comprise *luxA, luxB, luxC, luxD* and *luxE*; claim 1), and "polynucleotide sequences comprising Gram-positive ribosome-binding site sequences are located adjacent the 5' end of the luxA coding sequences, adjacent the 5' end of the luxB coding sequences, and adjacent the 5' end of the luc coding sequences" (claim 21). As noted in the specification, the sources of the *luxA, luxB, luxC, luxD* and *luxE* genes are Gram-negative organisms (e.g., Specification, page 1, lines 8-24).

The cited references must teach or suggest all of the claim limitations (see, e.g., M.P.E.P. 2143, Eighth Edition).

The cited references (Meighen, Vellanoweth, et al., and Schauer) considered alone or in combination, do not teach or suggest all of the limitations of the invention as presently claimed. Accordingly, for independent claims 1 and 21, as well as all dependent claims, the Examiner has not met the burden of establishing a *prima facie* case of obviousness. In view of the above-presented arguments, applicants submit that the rejection of the claims under 35 U.S.C. §103(a) is inappropriate and should be withdrawn.

C. The Examiner Has Improperly Combined The Teachings Of The Cited References.

In order to render the claims obvious, the burden is on the Examiner to establish a *prima facie* case of obviousness for which three basic criteria must be met. According to M.P.E.P. 2143 (Eighth Edition):

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.
>
> The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

As noted above, applicants submit that the cited references do not teach or suggest all the limitations of the present invention. However, even if, for the sake of argument, the elements of the present invention were taught by the cited references, applicants submit

that (i) there is no suggestion or motivation in the prior art to combine the references as suggested by the Examiner, and (ii) there is no reasonable expectation in the prior art that the combination suggested by the Examiner would be successful.

The cited combination of references is based only on impermissible hindsight reconstruction. As the court stated in *In re Vaeck*, cited above, the teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicants' disclosure. The Examiner has provided no motivation to combine the references other than the Examiner's speculation that one of ordinary skill in the art would be motivated to combine different teachings from the cited references, for example:

> It would have been obvious to one of ordinary skill in the art at the time the invention was made to use Gram-positive RBS in cassettes comprising the entire *lux* operon and intended for transforming Gram-positive bacteria as taught by Vellanoweth et al. (Office action, page 9, first full paragraph.)
>
> It would have been obvious to one of ordinary skill in the art at the time the invention was made to use Gram-positive RBS in cassettes comprising luxAB and luc genes and intended for transforming Gram-positive bacteria as taught by Vellanoweth et al. (Office action, pages 9-10, bridging paragraph).
>
> In order to avoid addition of an exogenous aldehyde, it would have been obvious to one of ordinary skill in the art at the time the invention was made to express the lux genes encoding both the luciferase and the substrate using genes taught by Meighen et al. (Office action, page 10, third full paragraph.)

The Examiner has provided no evidence to support these assertions of obviousness. The specific teachings of Vellanoweth, et al., and Meighen are addressed below. None of the cited references teaches the properties of the presently claimed invention. Meighen teaches only the organization, regulation, and general applications of *lux* genes. Meighen states:

> As the *lux* proteins have been expressed in a number of different gram-negative and gram-positive bacteria as well as in some eukaryotic organisms, general problems related to the stability of luciferases in recombinant cells are not anticipated. (Meighen, page 1020, col. 2, second full paragraph.)

Meighen contains no teaching or suggestion that it is desirable to prepare a polynucleotide encoding *luxA, luxB, luxC, luxD* and *luxE* gene products, wherein (a) transcription of the polynucleotide results in a polycistronic RNA encoding all the gene products; (b) each of the *luxA, luxB, luxC, luxD* and *luxE* gene products is expressed as an individual polypeptide; and (c) polynucleotide sequences comprising Gram-positive ribosome-binding site sequences are located

5' to all of said *lux* coding sequences (independent claim 1).

The abstract of Vellanoweth, et al., contains no teachings or suggestions that make up for the shortcomings of Meighen as a primary reference. In the rejection the Examiner asserts the following:

> Vellanoweth et al. teach that Gram-positive ribosomes are unable to translate mRNA containing weak Gram-negative RBS. (Office action, page 8, last paragraph.)

Applicants do not see where in the abstract the Examiner finds support for this assertion. The abstract of Vellanoweth, et al., teaches the following:

> The degree of emphasis placed on initiation codon type, as measured by translational yield, was dependent on the strength of the Shine-Dalgarno interaction in both organisms. B. subtilis was also much less able to tolerate secondary structure in the RSB than E. coli. While significant differences were found between the two organisms in the effect of specific RBS elements on translation, other mRNA components in addn. to those elements tested appear to be responsible, in part, for translational species specificity. (Vellanoweth, et al., Abstract.)

The source of the RSB (i.e., gram-positive bacteria, gram-negative bacteria or both) is not even clear from the abstract. As noted in Vellanoweth, et al.:

> The approach was used to analyze systematically the influence of spacing between the Shine-Dalgarno sequence and the initiation codon, the three different initiation codons, and RBS secondary structure on the translational yields in the two organisms. (Vellanoweth, et al., Abstract.)

There is no motivation from this abstract to modify a *lux* operon (derived from a Gram-negative organism) with RBS sequences from a Gram-positive organism according to the teachings of the present invention.

Regarding independent claim 21, similar arguments apply as set forth above for claim 1. In this case, the primary reference (Schauer) appears to teach a fusion of *luxA* and *luxB* encoded gene products. The reference separately teaches use of the *luc* gene as a reporter. There is no teaching or suggestion in the primary reference (Schauer) or in either secondary reference (Vellanoweth, et al. and Meighen) for a polynucleotide encoding *luxA, luxB*, and *luc* gene products, wherein (a) transcription of the polynucleotide results in a polycistronic RNA encoding all three gene products, (b) polynucleotide sequences comprising Gram-positive ribosome-binding

site sequences are located adjacent the 5' end of the luxA coding sequences, adjacent the 5' end of the luxB coding sequences, and adjacent the 5' end of the luc coding sequences, and (c) each of the *luxA, luxB,* and *luc* gene products is expressed as an individual polypeptide (independent claim 21). In claim 21 it is explicitly recited that each of the *luxA, luxB,* and *luc* gene products is expressed as an individual polypeptide (i.e., that *luxA* and *luxB* are not "fused"). In fact, the teaching of a *luxAB* fusion by Schauer teaches away from the use of genes separately encoding the *luxA* gene product and the *luxB* gene product, as in the present invention.

As stated by the Court of Appeals for the Federal Circuit *In re Fine*, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988):

> One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention.

Applicants submit that the Examiner has not given fair weight to what each reference teaches in its entirety. The Examiner has not addressed the differences between the prior art and the claims at issue. *Graham v. John Deere Co.*, 383 USC 1, 86 S. Ct. 684, 15 L Ed2d 545, 148 USPQ 459, S.C., 1966.

Obviousness cannot be established by combining teachings in the prior art absent some teaching or suggestion in the prior art that the combination be made. *In re Stence*, 828 F. 2d 751, 4 USPQ2d 1071 (Fed. Cir. 1987). *In re Newell,* 891 F. 2d 899, 13 USPQ2d 1248 (Fed Cir 1989). In particular, the fact that references can be combined does not make the combination obvious unless the prior art also contains something to suggest the desirability of that combination. *In re Sernaker*, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983).

In view of the above, all of the elements of the independent claims (1 and 21) are not taught by the cited references, no motivation to combine is found in the cited references, and no expectation of success is found in the cited references. Accordingly, applicants respectfully request that the rejections under 35 U.S.C. §103 be withdrawn.

D. Many Elements of the Dependent Claims Are Not Taught By The Cited Art.

Applicants submit that the Examiner has failed to establish a case of *prima facie* obviousness for the independent claims (claims 1 and 21) for the reasons discussed above. Further, the Examiner has not addressed many of the elements found in the dependent claims. For

example, no art has been cited to teach the following limitations of the dependent claims: claim 3, a Gram-positive ribosome binding site comprises the sequence presented as SEQ ID NO:1; claims 6 and 25, said promoter is contained in an Expression Enhancing Sequence selected from the group ~~consisting~~ of Sa1, Sa2, Sa3, Sa4, Sa5, and Sa6; claims 7 and 26, said promoter is contained in an Expression Enhancing Sequence selected from the group consisting of Sp1, Sp5, Sp6, Sp9, Sp16 and Sp17; claims 8 and 27, said promoter is contained in Expression Enhancing Sequence Sp16; claims 34 and 70, wherein the expression cassette is contained within a bacterial transposon; claims 35 and 71, wherein the expression cassette is contained within a bacterial mini-transposon; claims 36 and 72, wherein the coding sequences of the gene products comprise codons that are optimal for expression of the gene products in a host system into which the expression cassette is to be introduced; claims 49 and 73, a shuttle vector comprising, an expression cassette according to claim 1 (or 21), a polynucleotide encoding a selectable marker, a Gram-positive origin of replication, and a Gram-negative origin of replication; and claims 64 and 74, a Gram-positive bacteria comprising an expression cassette according to claim 1.

This list is not exhaustive, merely illustrative of claim limitations of the present invention that have not been addressed by the Examiner. Applicants respectfully request, if the rejection of the claims is to be maintained, that the Examiner address all of the recited claim limitations in any future rejections.

CONCLUSION

Applicants expressly reserve their right under 35 U.S.C. §121 to file one or more divisional applications directed to the non-elected subject matter during the pendency of this application. Upon allowance of generic claims, applicants request consideration of claims to additional species which are written in dependent form or which otherwise include all the limitations of the allowed generic claims (for example, claims 60 and 83-86).

Applicants respectfully submit that the claims comply with the requirements of 35 U.S.C. §112 and define an invention that is patentable over the art. Accordingly, a Notice of Allowance is believed in order and is respectfully requested.

If the Examiner notes any matters that may be facilitated by a telephone interview, applicants request that the Examiner contact the undersigned at the telephone number given below.

Please direct all further communications in this application to:

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Respectfully submitted,

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APPENDIX A

Techniques for determining nucleic acid and amino acid "sequence identity" also are known in the art. Typically, such techniques include determining the nucleotide sequence of the mRNA for a gene and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff, Atlas of Protein Sequences and Structure, M.O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, Nucl. Acids Res. 14(6):6745-6763 (1986). An exemplary implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics Computer Group (Madison, WI) in the "BestFit" utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, WI). A preferred method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases

= non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: http://www.ncbi.nlm.gov/cgi-bin/BLAST.

Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 80%-85%, preferably at least about 90%, and most preferably at least about 95%-98% sequence identity over a defined length of the molecules, as determined using the methods above. As used herein, substantially homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook et al., *supra*; *DNA Cloning*, *supra*; *Nucleic Acid Hybridization*, *supra*.

A "gene" refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular polypeptide or protein after being transcribed or translated. Any of the polynucleotide sequences described herein may be used to identify larger fragments or full-length coding sequences of the genes with which they are associated. Methods of isolating larger fragment sequences are known to those of skill in the art.

Two nucleic acid fragments are considered to "selectively hybridize" as described herein. The degree of sequence identity between two nucleic acid molecules affects the efficiency and strength of hybridization events between such molecules. A partially identical nucleic acid sequence will at least partially inhibit a completely identical sequence from hybridizing to a target molecule. Inhibition of hybridization of the completely identical sequence can be assessed using hybridization assays that are well known in the art (e.g., Southern blot, Northern blot, solution hybridization, or the like, see Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, (1989) Cold Spring Harbor, N.Y.). Such assays can be conducted using varying degrees of selectivity, for example, using conditions varying from low to high stringency. If conditions of low stringency are employed, the absence of non-specific binding can be assessed using a secondary probe that

Marked-up version showing amendments made to the claims by this paper.

1. An expression cassette comprising,

a polynucleotide encoding *luxA, luxB, luxC, luxD* and *luxE* gene products, wherein (a) transcription of the polynucleotide results in a polycistronic RNA encoding all the gene products; (b) each of the *luxA, luxB, luxC, luxD* and *luxE* gene products is expressed as an individual polypeptide; and (c) polynucleotide sequences comprising Gram-positive ribosome-binding site sequences are located 5' to all of said *lux* coding sequences.

2. The expression cassette of claim 1, further comprising a multiple-insertion site located 5' to said *luxA, luxB, luxC, luxD* and *luxE* coding sequences.

3. The expression cassette of claim 1, wherein at least one Gram-positive ribosome binding site comprises the sequence presented as SEQ ID NO:1.

4. The expression cassette of claim 1, wherein the coding sequences of the gene products are derived from *Photorhabdus luminescens*.

5. The expression cassette of claim 1, wherein the polynucleotide further comprises a promoter located 5' to all of said *lux* coding sequences wherein transcription of the polynucleotide results in a polycistronic RNA encoding all the *lux* gene products.

6. The expression cassette of claim 5, wherein said promoter is contained in an Expression Enhancing Sequence selected from the group consisting of Sa1, Sa2, Sa3, Sa4, Sa5, and Sa6.

7. The expression cassette of claim 5, wherein said promoter is contained in an Expression Enhancing Sequence selected from the group consisting of Sp1, Sp5, Sp6, Sp9, Sp16 and Sp17.

8. The expression cassette of claim 7, wherein said promoter is contained in Expression Enhancing Sequence Sp16.

21. An expression cassette comprising,

a polynucleotide encoding *luxA, luxB*, and *luc* gene products, wherein (a) transcription of the polynucleotide results in a polycistronic RNA encoding all three gene products, (b) polynucleotide sequences comprising Gram-positive ribosome-binding site sequences are located adjacent the 5' end of the luxA coding sequences, adjacent the 5' end of the luxB coding sequences, and adjacent the 5' end of the luc coding sequences, and (c) each of the *luxA, luxB,* and *luc* gene products is expressed as an individual polypeptide.

22. The expression cassette of claim 21, wherein said polynucleotide further encodes *luxC, luxD* and *luxE* gene products, wherein (i) Gram-positive ribosome-binding site sequences are located 5' to each of the *luxC, luxD,* and *luxE* coding sequences, and (ii) each of the *luxC, luxD, and luxE* gene products is expressed as an individual polypeptide.

24. The expression cassette of claim 21, wherein the polynucleotide further comprises a promoter located 5' to all of said *lux* and *luc* coding sequences wherein transcription of the polynucleotide results in a polycistronic RNA encoding all the *lux* and *luc* gene products.

25. The expression cassette of claim 24, wherein said promoter is contained in an Expression Enhancing Sequence selected from the group consisting of Sa1, Sa2, Sa3, Sa4, Sa5, and Sa6.

26. The expression cassette of claim 24, wherein said promoter is contained in an Expression Enhancing Sequence selected from the group consisting of Sp1, Sp5, Sp6, Sp9, Sp16 and Sp17.

27. The expression cassette of claim 26, wherein said promoter is contained in Expression Enhancing Sequence Sp16.

28. The expression cassette of claim 21, further comprising a multiple-insertion site located 5' to said *luxA, luxB, luc, luxC, luxD* and *luxE* coding sequences.

29. The expression cassette of claim 21, wherein the coding sequences for *luxA* and *luxB* are obtained from *Photorhadus luminescens.*

34. The expression cassette of claim 1, wherein the expression cassette is contained within a bacterial transposon.

35. The expression cassette of claim 1, wherein the expression cassette is contained within a bacterial mini-transposon.

36. The expression cassette of claim 1, wherein the coding sequences of the gene products comprise codons that are optimal for expression of the gene products in a host system into which the expression cassette is to be introduced.

49. A shuttle vector comprising:
an expression cassette according to claim 1;
a polynucleotide encoding a selectable marker;
a Gram-positive origin of replication; and
a Gram-negative origin of replication.

56. A method of modifying a Gram-positive organism to produce light, comprising transforming the Gram-positive organism with an expression cassette according to claim 1.

58. A method of screening an analyte for its ability to affect expression of a reporter marker, comprising:

providing the analyte to Gram-positive bacteria comprising the luciferase expression cassette of claim 1, wherein said reporter marker comprises luciferase; and

monitoring the effect of the analyte on the ability of the Gram-positive bacteria to produce light, thereby identifying whether the analyte affects expression of the reporter in Gram-positive bacteria.

60. (Withdrawn from consideration) A method of screening an analyte for its ability to affect expression of a reporter marker in a living, non-human animal, comprising:

introducing Gram-positive bacteria comprising the luciferase expression cassette of claim 1 into the animal, wherein said reporter marker comprises luciferase;

providing the analyte to the animal; and

monitoring the effect of the analyte on the ability of the Gram-positive bacteria to produce light, thereby identifying whether the analyte affects expression of the reporter in Gram-positive bacteria in the living, non-human animal.

62. (Canceled) A Gram-positive bacteria capable of producing light, wherein (a) the bacteria comprises luxA, luxB, luxC, luxD, and luxE coding sequences, and (b) about 1 x 10^6 bacterial cells can produce at least about 1 x 10^4 Relative Light Units at about 37°C.

64. A Gram-positive bacteria comprising an expression cassette according to claim 1.

68. The expression cassette of claim 1, wherein the arrangement of the coding sequences for the *lux* gene products is in the following relative order 5' - *luxA-luxB-luxC-luxD-luxE-* 3'.

69. The expression cassette of claim 21, wherein the arrangement of the coding sequences for the *lux* gene products is in the following relative order 5' - *luxA-luxB-luxC-luxD-luxE-* 3'.

70. The expression cassette of claim 21, wherein the expression cassette is contained within a bacterial transposon.

71. The expression cassette of claim 21, wherein the expression cassette is contained within a bacterial mini-transposon.

72. The expression cassette of claim 21, wherein the coding sequences of the gene products comprise codons that are optimal for expression of the gene products in a host system into which the expression cassette is to be introduced.

73. A shuttle vector comprising:
an expression cassette according to claim 21;
a polynucleotide encoding a selectable marker;
a Gram-positive origin of replication; and
a Gram-negative origin of replication.

74. A Gram-positive bacteria comprising an expression cassette according to claim 21.

75. A bacteria comprising the vector of claim 49.

76. A bacteria comprising the vector of claim 73.

77. A method of modifying a Gram-positive organism to produce light, comprising transforming the Gram-positive organism with an expression cassette according to claim 21.

78. The method of claim 77 further comprising providing the substrate required for *luc*-mediated luciferase activity.

79. (Amended) A method of screening an analyte for its ability to affect expression of a reporter marker, comprising:

providing the analyte to Gram-positive bacteria comprising the luciferase expression cassette of claim 21, wherein said reporter marker comprises luciferase;

providing one or more substrates required for luciferase light production; and

monitoring the effect of the analyte on the ability of the Gram-positive bacteria to produce light, thereby identifying whether the analyte affects expression of the reporter in Gram-positive bacteria.

80. (Amended) The method of claim 79, wherein said one or more substrates [is] comprise aldehyde [and is] provided as a vapor.

81. (Amended) The method of claim 79, wherein said one or more substrates [is] comprise a substrate for the *luc* gene product.

82. (Amended) The method of claim 79, wherein said one or more substrates [is] comprise (i) aldehyde, [and is] provided as a vapor, and (ii) a substrate for the *luc* gene product.

83. (Withdrawn from consideration) A method of screening an analyte for its ability to affect expression of a reporter marker in a living, non-human animal, comprising:

introducing Gram-positive bacteria comprising the luciferase expression cassette of claim 21 into the animal, wherein said reporter marker comprises luciferase;

providing the analyte to the animal;

providing substrate required for luciferase light production; and

monitoring the effect of the analyte on the ability of the Gram-positive bacteria to produce light, thereby identifying whether the analyte affects expression of the reporter in Gram-positive bacteria in the living, non-human animal.

84. (Withdrawn from consideration) The method of claim 83, wherein said substrate is aldehyde and is provided by injection.

85. (Withdrawn from consideration) The method of claim 83, wherein said substrate is a substrate for the luc gene product and is provided by injection.

86. (Withdrawn from consideration) The method of claim 83, wherein said substrate is (i) aldehyde and is provided as a vapor, and (ii) a substrate for the luc gene product.

APPENDIX B

Techniques for determining nucleic acid and amino acid "sequence identity" also are known in the art. Typically, such techniques include determining the nucleotide sequence of the mRNA for a gene and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff, Atlas of Protein Sequences and Structure, M.O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, Nucl. Acids Res. 14(6):6745-6763 (1986). An exemplary implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics Computer Group (Madison, WI) in the "BestFit" utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, WI). A preferred method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases

= non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: www.ncbi.nlm.gov/cgi-bin/BLAST.

Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 80%-85%, preferably at least about 90%, and most preferably at least about 95%-98% sequence identity over a defined length of the molecules, as determined using the methods above. As used herein, substantially homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook et al., *supra*; *DNA Cloning*, *supra*; *Nucleic Acid Hybridization*, *supra*.

A "gene" refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular polypeptide or protein after being transcribed or translated. Any of the polynucleotide sequences described herein may be used to identify larger fragments or full-length coding sequences of the genes with which they are associated. Methods of isolating larger fragment sequences are known to those of skill in the art.

Two nucleic acid fragments are considered to "selectively hybridize" as described herein. The degree of sequence identity between two nucleic acid molecules affects the efficiency and strength of hybridization events between such molecules. A partially identical nucleic acid sequence will at least partially inhibit a completely identical sequence from hybridizing to a target molecule. Inhibition of hybridization of the completely identical sequence can be assessed using hybridization assays that are well known in the art (e.g., Southern blot, Northern blot, solution hybridization, or the like, see Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, (1989) Cold Spring Harbor, N.Y.). Such assays can be conducted using varying degrees of selectivity, for example, using conditions varying from low to high stringency. If conditions of low stringency are employed, the absence of non-specific binding can be assessed using a secondary probe that